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(54) Title: A METHOD FOR THE IDENTIFICATION OF COMPOUNDS WITH ANXIOLYTIC POTENTIAL

(57) Abstract

The present invention relates to the use of a cloned GABA<sub>A</sub> receptor subtype composed of the  $\alpha_2$ ,  $\beta_3$  and  $\gamma_2$  subunits for the identification of benzodiazepine receptor ligands with selective anxiolytic properties (i.e. non-sedative anxiolytics). Affinity and/or efficacy of the ligands are measured at different cloned GABA<sub>A</sub> receptor subtypes.

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## A METHOD FOR THE IDENTIFICATION OF COMPOUNDS WITH ANXIOLYTIC POTENTIAL

### TECHNICAL FIELD

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The present invention relates to the use of a cloned GABA<sub>A</sub> receptor subtype composed of the  $\alpha_2$ ,  $\beta_3$  and  $\gamma_2$  subunits for the identification of benzodiazepine receptor ligands with selective anxiolytic properties (i.e. non-sedative anxiolytics).

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### BACKGROUND ART

Receptors for the major inhibitory neurotransmitter,  $\gamma$ -aminobutyric acid (GABA), are divided into two main classes, GABA<sub>A</sub> receptors and GABA<sub>B</sub> receptors.

GABA<sub>A</sub> receptors are members of a ligand-gated ion channel family, and 15 are the most abundant inhibitory receptors in the mammalian brain. Each GABA<sub>A</sub> receptor complex comprises a chloride ion channel that controls the chloride flux across the neuronal membrane, along with multiple recognition sites for small modulatory molecules such as benzodiazepines, barbiturates, picrotoxin, and certain steroids. When GABA interacts with its receptor, the ion channel is opened, the 20 chloride flux is enhanced, and the cell becomes less responsive to excitatory stimuli. This GABA induced ion current can be regulated by various agents, including agents that interact with the benzodiazepine receptor or recognition site.

Agents that bind or interact with the modulatory sites on the GABA<sub>A</sub> receptor complex, e.g. the benzodiazepine receptor, and have a positive modulatory 25 effect on the action of GABA, are called benzodiazepine receptor agonists or partial agonists. Agonists generally produce muscle relaxant, hypnotic, sedative, anxiolytic, and/or anticonvulsant effects.

Benzodiazepine receptor ligands with negative modulatory effect on the action of GABA are termed inverse agonists, while benzodiazepine receptor ligands 30 with no intrinsic activity are termed antagonists.

Numerous compounds belonging to different series of compounds having affinity for the benzodiazepine receptors have been synthesized during the last three decades. In particular it has been the aim of several groups to develop

benzodiazepine receptor modulators having potent anxiolytic activity and devoid of sedative effects. However, although the benzodiazepine receptor sites are still considered as very attractive biological sites for interfering with the CNS to treat various disorders and diseases, then nearly all previously synthesized compounds 5 acting at these receptor sites have failed during clinical development because of unacceptable side effects.

The  $\text{GABA}_A$  receptors are structurally constituted macromolecular heteropentameric assemblies, containing a combinations of  $\alpha$ ,  $\beta$ , and  $\gamma/\delta$  protein subunits. Several subunits of such  $\text{GABA}_A$  receptors ( $\alpha_{1-6}$ ,  $\beta_{1-3}$ ,  $\delta$  and  $\gamma_{1-3}$ ) have been 10 characterized using techniques of modern molecular biology. Considering the number of individual mammalian  $\text{GABA}_A$  receptor subunits, the number of pentameric subtypes with different subunit permutations that theoretically could exist, is overwhelming. It has therefore been a major challenge to delineate the subunit complements of native  $\text{GABA}$  receptors and to resolve their physiological role.

15 It is believed that specific  $\text{GABA}_A$  receptor subtypes are responsible for mediating the anxiolytic effect of benzodiazepines. But the specific subtypes and/or subunits involved in the patophysiology of anxiety disorders is not known at present.

From anatomical/behavioral studies in rats it has been shown that the anti-conflict effect of the benzodiazepine Midazolam<sup>TM</sup> is mediated at the baso-lateral 20 amygdaloid nucleus (European Journal of Pharmacology, 1982 **82** 115-116, and Neuroscience Letters, 1985 **53** 285-288). As mentioned above six  $\alpha$  subunits exists and benzodiazepines are believed to bind to  $\alpha$ -subunits or at the interface between the  $\alpha$  subunit and the  $\beta$  subunit. The  $\alpha_1$  subunit is distributed more or less uniformly throughout the rat brain, and is believed to be involved in the sedative affects of 25 benzodiazepine receptor ligands. The affinity of benzodiazepines for  $\alpha_4$  and  $\alpha_6$  subunits is low and it is therefore highly unlikely that benzodiazepines mediate their effects via these subunits.

From anatomical studies with *in situ* hybridization it has been shown that  $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$ ,  $\alpha_4$  and  $\alpha_5$  subunits are present in the baso-lateral amygdaloid nucleus,  $\alpha_2$  being 30 the most abundant  $\alpha$ -subunit (The Journal of Neuroscience, 1992 **12** (3) 1040-1062).

Additionally, It has been shown that the  $\beta_3$  subunit is the most abundant  $\beta$  subunit in the baso-lateral amygdaloid nucleus and that the  $\beta_3$  subunit seems

predominantly to be expressed in combination with  $\alpha_2$  subunits (The Journal of Neuroscience, 1992 12 (3) 1040-1062, and Biochem. J. 1995 310 1-9). Likewise the most abundant  $\gamma$  subunit in the baso-lateral amygdaloid nucleus is the  $\gamma_2$  subunit.

These findings suggests that the  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor subtype may be 5 the sole mediator of the anxiolytic effect of benzodiazepines.

By measuring the affinity and efficacy of a novel benzodiazepine receptor agonist having potent anxiolytic properties and low sedative effects on cloned GABA<sub>A</sub> receptor subtypes it was found that this novel benzodiazepine receptor ligand is a full agonist to the  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor subtype and a partial agonist the  $\alpha_1\beta_2\gamma_2$  GABA<sub>A</sub> receptor subtype. 10

Based on these findings a novel method for the identification of compounds with anxiolytic potential have been developed.

## SUMMARY OF THE INVENTION

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It is an object of the present invention to provide a method for the identification of benzodiazepine receptor ligands with selective anxiolytic properties (i.e. non-sedative) by measuring the affinity and/or efficacy of the test compounds to a cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor and comparing with the affinity and/or efficacy of the 20 test compound to other GABA<sub>A</sub> receptor subtypes which are known not to mediate selective anxiolytic effects. The present invention provides a valuable tool for screening chemical libraries for compounds with selective anxiolytic activity and for designing drugs with selective anxiolytic activity.

Accordingly, in its first aspect, the invention provides a method for the 25 identification of chemical compounds having anxiolytic potential and no or only low sedative effects, which method comprises the steps of

- 30 (i) measuring the affinity and/or efficacy of a chemical compound at a cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor;
- (ii) comparing the affinity and/or efficacy measured in step (i) with the affinity and/or efficacy of the same chemical compound at a different cloned GABA<sub>A</sub> receptor subtype; and
- (iii) selecting chemical compounds which are selective benzodiazepine receptor agonists at the  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor subtype.

In another aspect the invention provides a chemical compound having anxiolytic potential obtained by the method of the invention.

In a third aspect the invention provides a chemical compound being a selective benzodiazepine receptor agonists at the  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor subtype.

5 In further aspects the invention relates to the use of a chemical compound  
of the invention for use as a medicament, and to pharmaceutical compositions  
comprising these compounds.

Finally the invention relates to a method for the treatment of an individual suffering from anxiety comprising administering to said individual a therapeutically effective amount of a compound which is a selective benzodiazepine receptor agonist at the  $\alpha_2\beta_3\gamma_2$  GABA receptor.

## **DETAILED DISCLOSURE OF THE INVENTION**

15 The present invention provides for the identification of chemical compounds having potential as benzodiazepine receptor ligands with anxiolytic activity and no or only low sedative effects.

The method of the invention comprises measuring the affinity and/or efficacy of a chemical compound (the test compound) for binding onto a cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor, and comparing the affinity and/or efficacy so determined with the affinity and/or efficacy of the same compound for binding onto another cloned GABA<sub>A</sub> receptor subtype. In this way compounds which are selective benzodiazepine receptor agonists at the  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor subtype can be identified.

As defined herein, selective affinity for a GABA<sub>A</sub> receptor means that the affinity of a compound for the receptor is higher than the affinity of the compound to other GABA<sub>A</sub> receptors.

Also, as defined herein, a selective agonist of a GABA<sub>A</sub> receptor is a positive modulator of a GABA<sub>A</sub> receptor which have a higher efficacy to this receptor than to other GABA<sub>A</sub> receptors.

30 In a preferred embodiment, the method of the invention comprises  
(i)  $\text{m}_1$

(i) measuring the affinity of test compounds to cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptors and to other cloned GABA<sub>A</sub> receptor subtypes;

- (ii) selecting compounds with selective affinity for the cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptors;
- (iii) measuring the efficacy of the selected compounds at the cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor; and
- 5 (iv) selecting compounds which are selective agonist at the cloned GABA<sub>A</sub> receptor.

In another preferred embodiment the method of the invention comprises

- (i) measuring the affinity of test compounds to a cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor and to other cloned GABA<sub>A</sub> receptor subtypes;
- 10 (ii) selecting compounds with affinity for the cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor;
- (iii) measuring the efficacy of the selected compounds at the cloned GABA<sub>A</sub> receptors; and
- (iv) selecting compounds which are selective agonists at the cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor.

15 In the method of the invention, the affinity of the test compounds for the GABA<sub>A</sub> receptor subtypes may be determined by the ability of the test compounds to displace a radio labeled non-selective, high affinity benzodiazepine receptor ligand, such as tritium isotope radio labeled Flumazenil™ or Flunitrazepam™.

Alternatively, the efficacy of the test compounds for the GABA<sub>A</sub> receptor 20 subtypes may be determined by measuring the affinity of the compounds for the receptor in presence an in absence of GABA and calculating the GABA-ratio.

In yet another embodiment, the efficacy of the test compounds for the GABA<sub>A</sub> receptor may be determined by measuring the chloride flux using patch clamp technique.

25 In another preferred embodiment, the additional cloned GABA<sub>A</sub> receptor subtypes employed in the method of the invention, andnot being a cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor, are subtypes which are known not to mediate selective anxiolytic effects. More preferred this additional cloned GABA<sub>A</sub> receptor subtypes contain an  $\alpha_1$ ,  $\alpha_3$  or  $\alpha_5$  GABA<sub>A</sub> receptor subunit. In a most preferred embodiment, this additional 30 cloned GABA<sub>A</sub> receptor subtypes contain the subunit combination  $\alpha_1\beta_3\gamma_2$  or  $\alpha_3\beta_2\gamma_2$ .

In a particular embodiment of the invention, stabile CHO (Chinese hamster ovary) cells expressing cloned rat or human GABA receptor subunits is used to screen test compounds for their affinity to the  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor subtype. The affinity of

the test compounds to this receptor subtype is compared with the affinity of the compounds to GABA<sub>A</sub> receptor types which are believed not to mediate selective anxiolytic effects, e.g. GABA<sub>A</sub> receptor subtypes containing an  $\alpha_1$  receptor subunit. The affinity of the test compounds for the cloned receptor is determined by measuring 5 the ability of the compounds to displace a non-selective radio labeled benzodiazepine receptor ligand, such as tritium isotope radio labeled Flumazenil™ or Flunitrazepam™. Inhibition of binding is measured by a conventional in vitro binding assays using membrane preparations of cells expressing cloned GABA<sub>A</sub> receptor subtypes.

The efficacy of the test compounds, i.e. whether the compounds are 10 agonists, inverse agonists or antagonists at the benzodiazepine receptor, can also be determined by calculating the GABA-ratio of the compounds for each of the receptor subtypes employed. GABA-ratio is the ratio between the affinity of a test compound measured in presence of GABA and the affinity of the compound measured in absence of GABA. Benzodiazepine receptor ligands which are full agonists generally 15 have a GABA ratio between 1.8-3, partial agonists have a ratio between 1.0-1.8, antagonists have a ratio around 1, whereas inverse agonist have a GABA ratio around 0.5 -1. Calculation of GABA-ratio therefore provides an indication of the efficacy of the test compound.

The efficacy of the compound can also be measured in electrophysiological 20 experiments using patch clamp technique.

In the method of the invention any cell expressing GABA<sub>A</sub> receptor subunits may be employed. Preferred cells are CHO cells, or other rodent fibroblast cell lines such as BHK (baby kidney hamster cells) and mouse Ltk<sup>-</sup> cells; insects cells, such as Sf-9 cells, and HEK293 or HeLa cells. The GABA<sub>A</sub> subunits expressed by the cells 25 may be of animal (rat or bovine) or human origin.

In another aspect the invention provides a chemical compound having anxiolytic potential obtained by the method of the invention.

In a third aspect the invention provides a chemical compound being a selective benzodiazepine receptor agonists at the  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor subtype.

30 In further aspects the invention relates to the use of a chemical compound of the invention for use as a medicament, and to pharmaceutical compositions comprising these compounds.

Finally the invention relates to a method for the treatment of an individual suffering from anxiety comprising administering to said individual a therapeutically effective amount of a compound which is a selective benzodiazepine receptor agonist at the  $\alpha_2\beta_3\gamma_2$  GABA receptor.

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### EXAMPLES

The invention is further illustrated with reference to the following example which is not intended to be in any way limiting to the scope of the invention as claimed.

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The affinity of 5-acetyl-1-(3-(3-pyridyl)phenyl)benzimidazole O-ethyl oxime for various GABA<sub>A</sub> receptor subtypes was studied using recombinant GABA<sub>A</sub> receptors expressed in insect cells. The ratio between affinity for the compound in the absence and presence of GABA (GABA ratio) depended on the subunit combination of the 15 cloned receptors. The compound had a GABA ratio corresponding to a full agonist at the  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor subtype and a GABA ratio corresponding to a partial agonist at the  $\alpha_1\beta_2\gamma_2$  GABA<sub>A</sub> receptor subtype.

## CLAIMS

1. A method for the identification of a chemical compound having anxiolytic potential and no or only low sedative effects, which method comprises the steps  
5 of

- (i) measuring the affinity and/or efficacy of a chemical compound at cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptors;
- (ii) comparing the affinity and/or efficacy measured in step (i) with the affinity and/or efficacy of the same chemical compound at different cloned GABA<sub>A</sub> receptor subtypes; and
- 10 (iii) selecting the chemical compound that is a selective benzodiazepine receptor agonist at the  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor subtypes.

2. The method according to claim 1, comprising

- 15 (i) measuring the affinity of the test compound to cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptors and to different cloned GABA<sub>A</sub> receptor subtypes;
- (ii) selecting the compound which has selective affinity for the cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptors;
- (iii) measuring the efficacy of the selected compound at the cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptors; and
- 20 (iv) selecting the compound which is a selective agonist at the cloned GABA<sub>A</sub> receptors.

3. The method according to claim 1, comprising

- 25 (i) measuring the affinity of the test compound to cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptors and to different cloned GABA<sub>A</sub> receptor subtypes;
- (ii) selecting the compound which has affinity for the cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptors;
- (iii) measuring the efficacy of the selected compound at the cloned GABA<sub>A</sub> receptors; and
- 30 (iv) selecting the compound which is a selective agonist at the cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptors.

4. The method according to any of claims 1-3, wherein the affinity of the test compound for the GABA<sub>A</sub> receptor subtypes is determined by measuring the ability of the test compound to displace a radio labeled non-selective, high affinity benzodiazepine receptor ligand, such as tritium isotope radio labeled Flumazenil™ or Flunitrazepam™.
5. The method according to any of claims 1-4, wherein the efficacy of the test compound for the GABA<sub>A</sub> receptor subtypes is determined by measuring the affinity of the compound for the receptors in the presence and in the absence of GABA, and calculating the GABA-ratio.
10. The method according to any of claims 1-4, wherein the efficacy of the test compound for the GABA<sub>A</sub> receptor subtypes is determined by measuring the chloride flux using patch clamp technique.
15. The method according to any of claims 1-6, wherein the cloned GABA<sub>A</sub> receptor subtypes that is different from the cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptors, are subtypes which are known not to mediate selective anxiolytic effects.
20. The method according to claim 1, wherein the cloned GABA<sub>A</sub> receptor subtypes that is different from the cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptors, contain the  $\alpha_1$ ,  $\alpha_3$  or  $\alpha_5$  GABA<sub>A</sub> receptor subunits.
25. The method according to claim 1, wherein the cloned GABA<sub>A</sub> receptor subtypes that is different from the cloned  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptors, contain the subunit combination  $\alpha_1\beta_3\gamma_2$  or  $\alpha_3\beta_2\gamma_2$ .
30. A chemical compound having anxiolytic potential obtained by the method according to any of claims 1-9.
11. A chemical compound being a selective benzodiazepine receptor agonists at the  $\alpha_2\beta_3\gamma_2$  GABA<sub>A</sub> receptor subtypes.

12. A pharmaceutical composition comprising the benzodiazepine receptor ligand according to either of claims 10-11.
- 5 13. The chemical compound according to either of claims 10-11 for use as a medicament.
14. Use of the chemical compound according to either of claims 10-11 for the manufacture of a medicament for the treatment of anxiety.

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15. A method for the treatment of an individual suffering from anxiety comprising administering to said individual a therapeutically effective amount of a chemical compound which is a selective benzodiazepine receptor agonist at the  $\alpha_2\beta_3\gamma_2$  GABA receptors.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/DK 97/00475

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: G01N 33/566, C12Q 1/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	WO 9413799 A1 (MERCK SHARP & DOHME LIMITED), 23 June 1994 (23.06.94), page 5, line 7 - line 10; page 11, example 4, claims 2,8-13  --	1-8,10-15
X,Y	TIPS, Volume 17, May 1996, Erminio Costa et al, "Benzodiazepines on trial: a research strategy for their rehabilitation", page 192 - page 200, see page 194 and page 196 - page 197, bridging paragraph  --	1-8,10-15

Further documents are listed in the continuation of Box C.

See patent family annex.

- \* Special categories of cited documents:
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- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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Date of the actual completion of the international search

Date of mailing of the international search report

07-02-1998

22 January 1998

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INTERNATIONAL SEARCH REPORTInternational application No.  
PCT/DK 97/00475

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, Y	Journal of biological chemistry, Volume 269, No 43, 1994, Dietmar Benke et al, "Distribution, Prevalence, and Drug Binding Profile of gamma-Aminobutyric Acid Type A Receptor Subtypes Differing in the beta-Subunit Variant", page 27100 - page 27107, see page 27105 - page 27106 and Table II --	1-5, 10-15
Y	WO 9322681 A1 (NEUROGEN CORPORATION), 11 November 1993 (11.11.93), page 4 - page 8, claim 1	1-5
X	--	10-15
Y	NeuroReport, Volume 6, No 3, February 1995, G. White et al, "alfa subunits influence Zn block of gamma2 containing GABA receptor currents", page 461 - page 464, figures 2,3 --	1-6
A	Dialog Information Services, File 154, MEDLINE, Dialog accession no. 07367310, Medline accession no. 92383385, Gardner CR: "A review of recently-developed ligands for neuronal benzodiazepine receptors and their pharmacological activities", Prog Neuropsychopharmacol Biol Psychiatry (England) 1992, 16 (6) p 755-81	1
X	--	10
A	US 5166066 A (DONALD B. CARTER), 24 November 1992 (24.11.92) --	1-6
A	The Journal of Neuroscience, Volume 12, No 3, March 1992, W. Wisden et al, "The Distribution of 13 GABA Receptor Subunit mRNAs in the Rat Brain. I. Telencephalon, Diencephalon, Mesencephalon" page 1040 - page 1062 --	1

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 97/00475

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>The Journal of Neuroscience, Volume 15, No 10, October 1995, Hartmut Lüddens et al, "GABA Antagonists Differentiate between Recombinant GABAA/Benzodiazepine Receptor Subtypes", page 6957 - page 6962, see Table 1, figures 1,2,4 and 5</p> <p>---</p> <p>-----</p>	1

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK97/00475

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: 15  
because they relate to subject matter not required to be searched by this Authority, namely:  
Claim 15 relates to a method of treatment of the human or animal body by surgery or by therapy/a diagnostic method practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for this claim. The search has been based on the alleged effects of the compound/composition.
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

07/01/98

International application No.

PCT/DK 97/00475

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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